AD-A202 171

REPORT DOCUMENTATION PAGE

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Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S): NMRI 88-59	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Naval Medical Research	6b. OFFICE SYMBOL (If applicable)
6c. ADDRESS (City, State, and ZIP Code): Bethesda, Maryland 20814-5055	7a. NAME OF MONITORING ORGANIZATION: Naval Medical Command
	7b. ADDRESS (City, State, and ZIP Code): Department of the Navy, Washington, D.C. 20372-5120
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: Naval Medical Research and Development Command	8b. OFFICE SYMBOL (If applicable)
8c. ADDRESS (City, State, and ZIP Code): Bethesda, Maryland 20814-5055	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. NA; PROJECT NO.; TASK NO.; WORK UNIT ACCESSION NO

11. TITLE (Include Security Classification)

Density gradients for isolation of mononuclear blood cells for magnesium analysis by electron probe x-ray microanalysis

12. PERSONAL AUTHOR(S) Hook GR, Hosseini JM, Elin RJ

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Journal article	FROM _____ TO _____	1988	5

16. SUPPLEMENTARY NOTATION

Reprinted from: Magnesium 1988 pp. 1-5 ; Vol. 7

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)

Monocuclear blood cells
Density gradient separation
Electron probe
Arabinogalactan

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC ELECTE DEC 3 0 1988 S D E

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Phyllis Blum, Information Services Division	22b. TELEPHONE (Include Area Code): 202-295-2188 / 22c. OFFICE SYMBOL: ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE: UNCLASSIFIED

88 12 20 060

Magnesium
Editor-in-Chief: B.M. Altura, Brooklyn, N.Y.
Associate Editor-in-Chief: J. Durlach, Paris

Reprint
Publisher: S. Karger AG, Basel
Printed in Switzerland

Original Papers

Magnesium 7: 1–5 (1988)

0252–1156/88/0071–0001$2.75/0

Density Gradients for Isolation of Mononuclear Blood Cells for Magnesium Analysis by Electron Probe X-Ray Microanalysis

Gregory R. Hook[a], *Jeanette M. Hosseini*[b], *Ronald J. Elin*[b]

[a] Surgical Neurology Branch, National Institutes of Neurological and Communicative Disorders and Stroke, and [b] Clinical Pathology Department, Clinical Center, National Institutes of Health, Bethesda, Md., USA

Key Words. Mononuclear blood cells · Density gradient separation · Electron probe · Arabinogalactan

Abstract. We present a mononuclear blood cell (MBC) isolation method for magnesium analysis by electron probe X-ray microanalysis. We compare the inorganic elemental composition of individual MBC isolated by either arabinogalactan (Stractan) or conventional Ficoll-Hypaque density gradients. We find that the MBC isolated with Stractan have the expected cellular element composition, but MBC isolated with Ficoll-Hypaque are contaminated with iodine and sodium. We discuss the source and significance of iodine and sodium contamination of Ficoll-Hypaque isolated MBC and recommend the Stractan separation method for electron probe X-ray microanalysis.

Introduction

Clinically, magnesium in peripheral mononuclear blood cells (MBC) may be a useful indicator of intracellular Mg status [1, 9]. Ficoll-Hypaque density gradient sedimentation is the standard method used to isolate MBC (primarily lymphocytes) from whole blood for experimentation. We initially used this method to prepare a MBC population for Mg analysis of individual whole cells using energy dispersive electron probe X-ray microanalysis (EPMA) [10] and immediately noted that the MBC were contaminated with iodine and sodium. To eliminate this problem, we isolated MBC using a discontinous arabinogalactan (Stractan) gradient. We present the Stractan MBC isolation method and compare EPMA spectra from whole cells isolated from Ficoll-Hypaque and Stractan media. We discuss the source of iodine and sodium contamination in Ficoll-Hypaque isolated cells and the effect of this contamination on analysis of Mg in MBC by EPMA.

Materials and Methods

Stractan MBC Isolation Method

Whole blood (10 ml) was collected from adult volunteers using sodium heparin as an anticoagulant. The blood was washed 3 times in isotonic, pH neutral, buffered saline glucose (BSG) and 1% bovine serum albumin (700 *g*, 3 min) to remove platelets. The buffy coat was collected with 1 ml of red blood cells and was brought up to a total volume of 4 ml with BSG (no bovine serum albumin). The buffy coat harvest was layered over a Stractan (St. Regis Paper Co., Tacoma, Wash.) discontinuous density gradient. The Stractan was purified by the method previously described [2]. The gradient was composed of an 18.2% Stractan in BSG (lower layer) and 13% Stractan in BSG (upper layer). Both Stractan solutions were at 4 °C and the gradient was centrifuged for 30 min at 2,000 *g* at room temperature [8]. The MBC were at the interface of the two Stractan solutions (fig. 1).

Ficoll-Hypaque MBC Isolation Method

MBC were isolated from whole blood as described for the Stractan method except a Ficoll-Hypaque gradient was used instead of the Stractan gradient. The Ficoll-Hypaque gradient was centrifuged for 40 min at 400 *g* at room temperature [3].

EPMA Sample Preparation

MBC isolated by Stractan or Ficoll-Hypaque gradients were washed in isotonic, pH 7.4 ammonium nitrate (NH_4NO_3) as previously described [6]. This solution maintains intact, viable lymphocytes during washing, yet forms a volatile salt that when dried is completely removed from the cells by the electron probe. The washed cells were spray-deposited on large-area thin film supports, air-dried and carbon-coated.

EPMA

Twenty cells from each preparation were analyzed. The X-ray spectra of individual cells were obtained using an electron microprobe (Cameca, Stamford, Conn.) and an energy dispersive detector with multichannel analyzer (Tracor Northern 5500, Middleton, Wisc.). The electron beam raster was adjusted so an individual cell was circumscribed by the scanned area. The electron beam accelerating voltage and current (Faraday cup measurement) were 35 kV and 20 nA, respectively, and a count rate of approximately 1,000 s was obtained.

Results

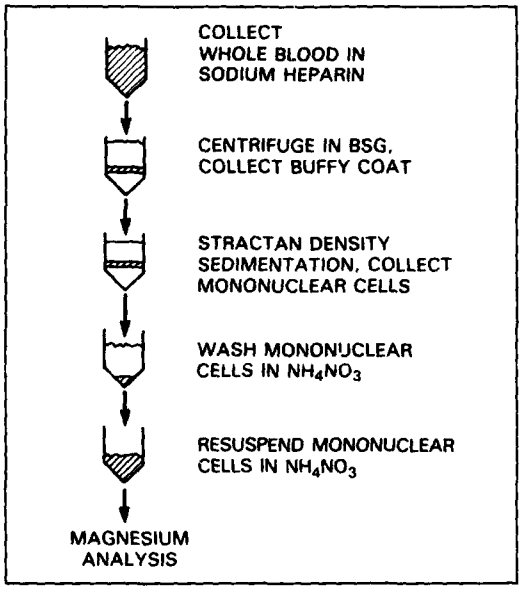
All Stractan isolated cells had only the elements expected to be present on or in the cells and did not have any exogenous elemental contamination (fig. 2). All of the Ficoll-Hypaque-isolated cells were contaminated with iodine and sodium (fig. 3) and some cells also had large amounts of calcium. No iodine was detected in the dried wash solution of MBC separated by Ficoll-Hypaque.

Discussion

Stractan is a polysaccharide composed of carbon, hydrogen and oxygen which are not detected by EPMA. Further, Stractan is probably removed from the MBC during washing because Stractan is very hydrophilic. In contrast, Hypaque contains iodine and sodium which contaminate the MBC. The chemical structure of sodium hypaque is shown in figure 4. The Hypaque probably binds to the lipid portion of the cell and is not removed by washing because the Hypaque benzene ring is hydrophobic.

Iodine and sodium contamination of MBC causes significant problems for the determination of in Mg by EPMA. Qualitatively, excessive exogenous sodium causes the tail of the sodium-characteristic X-ray peak to overlap with the Mg-characteristic X-ray peak. In addition, the iodine-characteristic X-ray peaks overlap with potassium- and calcium-characteristic X-ray peaks and iodine escape X-ray peaks overlap with phosphorus, sulfur and chlorine.

Quantitatively, heavy element contamination causes well known EPMA errors [4] in determining the Mg-characteristic peak-



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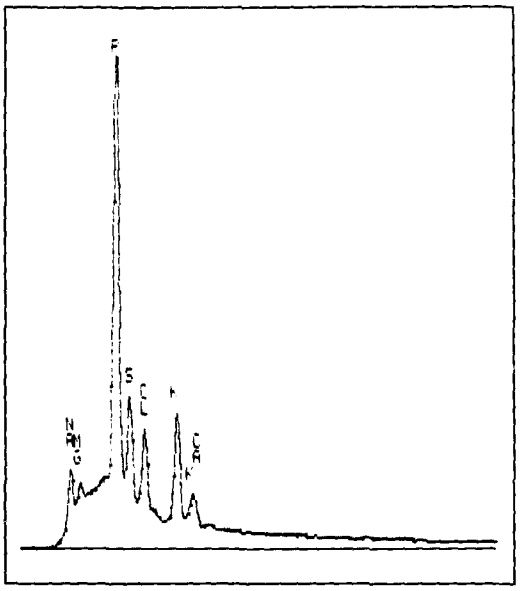

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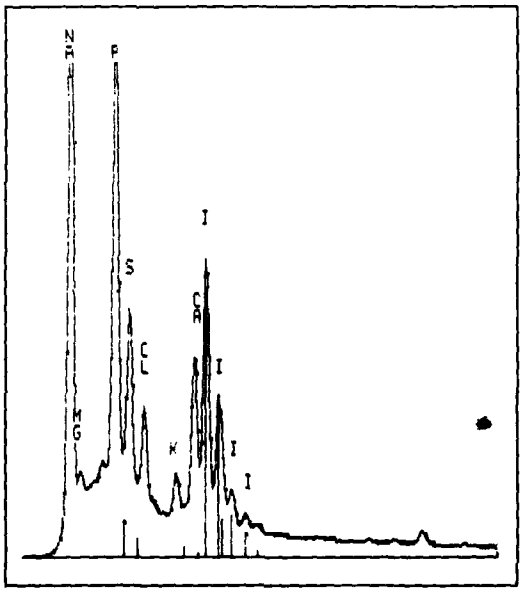
Fig. 1. The Stractan MBC isolation method is diagrammed.

Fig. 2. A typical EPMA X-ray spectrum from an individual MBC isolated by the Stractan method shows the presence of sodium, magnesium, phosphorus, sulfur chlorine, potassium and calcium. Stractan is composed of carbon, hydrogen and oxygen which are not detected by this method. The Stractan method is suitable for EPMA. The abscissa extends from 0 to 10.24 keV and the ordinate from 0 to 8,192 counts.



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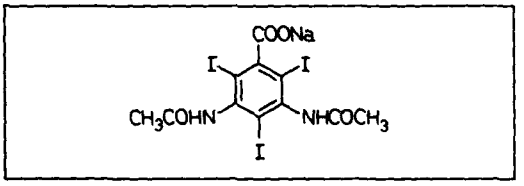

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Fig. 3. A typical EPMA X-ray spectrum from an individual mononuclear cell isolated by the Ficoll-Hypaque method shows the cell is contaminated with iodine and sodium. The contamination is due to the Hypaque and interferes with the analysis of Mg (see Discussion). The vertical bars on the spectrum show the expected positions and relative production efficiencies of the iodine characteristic and escape X-ray peaks. The Ficoll-Hypaque method is not suitable for EPMA. The abscissa extends from 0 to 10.24 keV and from the ordinate from 01 to 16,384 counts. The vertical full scale of figure 2 is half of figure 3.

Fig. 4. Structure of sodium hypaque.

to-continuum X-ray intensity ratio [5]. For samples on thin films, the characteristic X-ray intensity measures the elemental content, the continuum X-ray intensity measures the local mass and thus the characteristic peak-to-continuum X-ray intensity ratio measures the elemental concentration. However, continuum X-ray production is a function of atomic number and heavy elemental contamination will artifactually increase the continuum X-ray intensity. The increase in the continuum X-ray intensity by iodine will decrease the Mg peak-to-continuum X-ray intensity ratio and the measured MBC Mg concentration. Further, the amount of iodine may vary substantially among cells and introduce significant variability in quantitation of Mg.

In an earlier work, the MBC isolated from Stractan and Ficoll-Hypaque were compared for MBC Mg content, purity, yield, viability and ease of use [8]. The MBC Mg content was 78.4 ± 25.5 and 83.6 ± 30.8 (fg/cell) (mean ± SD) for the Stractan and Ficoll-Hypaque methods, respectively, and there was no statistically significant difference between these means. Likewise, there was no statistically significant difference for purity yield, and viability between the two methods. Ficoll-Hypaque is easier to use because it is commercially available and requires no preparative steps prior to use, whereas Stractan is not available ready-to-use and requires purification. Nonetheless, Stractan is a useful MBC separation gradient for EPMA. The Mg contentration of MBC isolated by the Stractan/NH_4 NO_3 method has been quantitated by EPMA and flame atomic absorption spectroscopy, MBC Mg contentrations of 64.0 ± 14.3 and 52.2 ± 13.8 mmol/kg dry weigth (mean ± SD), respectively, were found and there was no significant difference in the means [7]. In addition, washing lymphocytes in NH_4NO_3 and BSG solutions has been compared [6] and no significant difference was found for lymphocyte Mg, recovery, volume or viability. Thus, Stractan and NH_4NO_3 washing are reasonable sample preparatory procedures for EPMA of MBC Mg.

In conclusion, we describe a Stractan gradient for isolation of MBC which is suitable for EPMA. The Ficoll-Hypaque gradient for isolation of MBC is not suitable for EPMA because it results in iodine and sodium contamination which adhered to the cells after washing and interfered with EPMA analysis. The isolation of MBC by the Stractan method is recommended over the conventional Ficoll-Hypaque method for EPMA of Mg in MBC.

Acknowledgements

The authors wish to thank Ms. Dorene Dalessandro for her assistance in the preparation of the manuscript.

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Received: November 21, 1986
Accepted: September 8, 1987

Dr. Gregory Hook
Naval Medical Research Institute
Building 17
Bethesda, MD 20814 (USA)